Risk of Female Breast Cancer Associated with Serum Polychlorinated Biphenyls and 1,1-Dichloro-2,2′-bis(*p*-chlorophenyl)ethylene[1]

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Abstract

This case-control study was designed to investigate the relationship between polychlorinated biphenyls (PCBs) and 1,1-dichloro-2,2′-bis(*p*-chlorophenyl)ethylene (DDE) and breast cancer risk in Connecticut. Cases were incident breast cancer patients who were either residents of Tolland County or who had a breast-related surgery at the Yale-New Haven Hospital in New Haven County. Controls were randomly selected from Tolland County residents or from patients who had newly diagnosed benign breast diseases or normal tissue at Yale-New Haven Hospital. A total of 475 cases and 502 controls had their serum samples analyzed for PCBs and DDE in 1995–1997. The age- and lipid-adjusted geometric mean serum level of DDE was comparable between the cases (460.1 ppb) and controls (456.2 ppb). The geometric mean serum level of PCBs was also comparable between cases (733.1 ppb) and controls (747.6 ppb). After adjustment for confounding factors, odds ratios of 0.96 (95% confidence interval, 0.67–1.36) for DDE and 0.95 (95% confidence interval, 0.68–1.32) for PCBs were observed when the third tertile was compared with the lowest. Further stratification by parity, lactation, and menopausal and estrogen receptor status also showed no significant association with serum levels of DDE or PCBs. The results by PCB congener groups also showed no major increased risk associated with any of the congener groups. Our study does not support the hypothesis that DDE and PCBs, as encountered through environmental exposure, increase the risk of female breast cancer.

Introduction

Environmental exposure to organochlorine compounds, particularly PCBs,[3] DDT, and its most stable metabolite, DDE, recently have been suggested as risk factors for female breast cancer (1–4). It is conceivable that exposure to these environmental contaminants may increase breast cancer risk because some of the organochlorine compounds are animal carcinogens, estrogenically active, and inducers of cytochrome P-450 mixed-function oxidase enzymes, which are involved in steroid hormone metabolism (5–11).

Epidemiological studies linking PCB and DDE exposure to breast cancer risk, however, have produced inconclusive results. Among six follow-up studies that examined the relationship between PCBs and DDE and the risk of breast cancer, using a nested case-control study design, one found a dose-response relationship between breast cancer risk and serum DDE levels, and a possible threshold effect with serum PCB levels (12). Another study by Krieger *et al.* (13) suggested an increased risk of breast cancer associated with higher serum levels of DDE among Caucasian and African-American women. Four other recent studies, however, did not find an increased risk associated with serum levels of either DDE or PCBs (14–17).

Several pilot studies have assessed the relationship between levels of PCBs or DDE in adipose tissue and breast cancer risk (18–22). Four of the studies found higher adipose tissue levels of PCBs among breast cancer cases than non-cancer controls (18–21), and one suggested an increased risk of breast cancer associated with body levels of DDE in women with ER-positive breast cancer (22). Two recent larger case-control studies, one using breast adipose tissue (23) and the other using buttock adipose tissue (24), did not find a positive association between adipose tissue levels of DDE and DDT and breast cancer risk.

Three case-control studies that used blood drawn after the diagnosis of breast cancer assessed the risk of breast cancer associated with serum levels of PCBs or DDE (25–27). Although the studies conducted in North Vietnam (26) and Mexico (27) did not find an association between serum DDT and DDE levels and breast cancer risk, a study from upstate New York by Moysich *et al.* (25) did suggest an increased risk of breast cancer associated with serum levels of total PCBs among postmenopausal parous women who had never breast-fed an infant (OR = 2.9; 95% CI, 1.0–7.3).

Although recent epidemiological studies have not supported an overall association between PCBs and DDE exposure and breast cancer risk, several studies that have information on parity, lactation, and hormone receptor status seem to suggest

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[3] The abbreviations used are: PCB, polychlorinated biphenyl; DDT, 2,2′-bis(*p*-chlorophenyl)-1,1,1-trichloroethane; DDE, 1,1-dichloro-2,2′-bis(*p*-chlorophenyl)ethylene; ER, estrogen receptor; OR, odds ratio; CI, confidence interval; YNHH, Yale-New Haven Hospital; BMI, body mass index.

that reproductive factors and hormone receptor status may have an impact on the relationship between PCB and DDE exposures and subsequent development of breast cancer. To further address this issue, we report here the results from a case-control study that examined the relationship between serum levels of DDE and PCBs and breast cancer risk by menopausal status, parity and lactation, and by cases' hormone receptor status.

Materials and Methods

Study Subjects. Cases, recruited from 1995 to 1997, were histologically confirmed, incident breast cancer patients (International Classification of Diseases for Oncology, 174.0–174.9) who were either residents of Tolland County, or who had a breast related surgery at YNHH, in New Haven County, Connecticut. Cases and controls were 30–80 years of age, had no previous diagnosis of cancer, with the exception of non-melanoma skin cancer, were alive at the time of interview, and were willing to donate at least 10 ml of blood for organochlorine compound analyses.

Potentially eligible cases and controls from YNHH were identified using computerized patient information from the YNHH Surgical Pathology Department, where records of all newly completed breast-related surgeries are kept. We consecutively selected all breast cancer patients who met the study eligibility requirements as described above. A total of 326 incident breast cancer cases were recruited from YNHH. From the computerized files, we also randomly selected 347 controls who had had breast-related surgery and were histologically diagnosed with benign breast diseases. Efforts were made to frequency match the cases and controls by age within 5-year intervals (*e.g.*, 30–34, 35–39, 40–44) with a 1:1 ratio by adjusting the number of controls randomly selected in each age stratum every few months. Of the 347 YNHH controls, 37 subjects were diagnosed with normal tissue, 45 with fibroadenoma, 107 with other nonproliferative benign breast diseases, and 158 with proliferative benign breast diseases without atypia. Diagnoses of atypical hyperplasia were excluded. The participation rates were 71% for controls and 77% for cases among the YNHH patients.

In addition to the YNHH cases and controls drawn largely from New Haven County, we recruited cases and controls from Tolland County, Connecticut. The two counties have similar breast cancer incidence rates, and in recent years, also have had similar breast cancer mortality rates. Newly diagnosed cases with Tolland County addresses were identified from area hospital records by the Rapid Case Ascertainment Shared Resource of the Yale Cancer Center. A total of 149 cases were recruited. Population-based controls with Tolland County addresses were recruited using either random digit dialing methods for those below age 65 or from Health Care Finance Administration files for those age 65 and above. A total of 155 controls were recruited. Efforts were also made to frequency match the cases and controls by age within 5-year intervals with a 1:1 ratio by adjusting the number of controls randomly selected in each age stratum. The participation rates were 61% for controls and 74% for cases in Tolland County.

The study pathologist (D. C.) reviewed all of the pathological diagnoses for breast cancer patients and benign breast disease controls diagnosed at YNHH and also reviewed the pathology reports for the 149 cases ascertained from Tolland County. Carcinomas were classified as *in situ*, invasive ductal, or invasive lobular, and were staged according to the TNM system (28). For patients diagnosed at YNHH, we also collected information on ER levels, which were measured immunohistochemically at the Pathology Department of YNHH. ER status was considered positive when the H-score was higher than 75, as described by McCarty *et al.* (29). Treatment information for breast cancer patients was also collected from the Yale New Haven Hospital Tumor Registry, where computerized files contain information (including treatment data) for all cancer patients seen at YNHH.

Interviews. After approval by each subject's hospital and physician, potential participants were approached by letter and then by phone, and those who consented were interviewed by a trained interviewer, either in their homes or at locations convenient for the subjects. A standardized, structured questionnaire was used to obtain information on major known or suspected confounding factors, including menstrual and reproductive history, lactation history, past medical history, family cancer history, occupation, diet, and demographic factors. Dietary information was collected using a scannable semiquantitative food frequency questionnaire developed by the Fred Hutchinson Cancer Research Center, designed to optimize estimation of fat intake. Each subject was asked to characterize her usual diet in the year prior to being interviewed for our study. Following the interview, the participant provided a blood sample, collected by venipuncture by our study staff.

Blood Collection and Chemical Analysis. Blood samples were held in a cooler until serum was separated, usually within 1–3 h. The samples were then coded and stored in our study freezer at −84°C until they were sent in batches to the study laboratory at Colorado State University. All samples were kept frozen until analysis. Serum samples were analyzed in batches of 12, with each batch having ~5 cases, 5 controls, and 2 quality control samples. Laboratory personnel in Colorado were blind to the case-control status of samples being analyzed.

The analytical methods for determining PCB and DDE levels in 1.0 ml of serum have been described elsewhere (30). Briefly, the method involved denaturation of protein by methanol, extraction of the compounds of interest in ethyl ether: hexane (1:1, v/v), gravimetric lipid determination, purification of the sample using Florisil chromatography, and identification and quantification of the compounds by gas chromatography. Serum residue results are reported as ppb on a lipid adjusted basis. To obtain a lipid adjusted residue value, the wet weight value was divided by the serum's lipid content, and reported as nanograms of compound per gram of lipid.

The quantitation limit (the smallest amount of a compound that can be quantified consistently) by this method was 1.5 ppb for both PCBs and DDE. The detection limits of the method for PCBs and DDE were half of their quantitation limits. For DDE, 95% of the samples were above the detection limit. For PCBs, >70% of the samples were above the detection limit. No consistent way of dealing with values below the detection limit is given among the studies in the literature. We used two approaches: no correction for values below the detection limit, and correction for these values (substituting a hypothetical value equal to half of the detection limit for the assay for nondetectable subjects). Both of the analyses reached the same conclusion. Therefore, we present only the results with corrections. In this study, total PCBs was defined as the sum of the following measured PCB congeners: 74, 118, 138, 153, 156, 170, 180, 183, and 187. Total DDE was defined as the serum level of *p,p′*-DDE.

Strict quality control/quality assessment procedures were followed throughout sample analyses, including method spikes, reagent blanks, and quality control windows. Estimated recovery of the various analytes (including *p,p′*-DDE, and nine PCB

congeners) exceeded 95%, and the coefficients of variation for the various analytes were 9–15%.

Data Analysis. The primary analyses involved comparisons of serum levels of DDE and PCBs between all cases and controls. Because the distribution of PCBs and DDE was skewed, a log transformation was used to better approximate the normality assumption, and thus its antilog provided the geometric mean. The age-adjusted geometric means were found by analysis of covariance on the log exposure, and the antilog of the least squares means provided summary statistics. The statistical significance for the adjusted geometric means of serum levels of DDE and PCBs was determined using analysis of covariance.

Because earlier studies have suggested that environmental estrogens may affect only the incidence of hormone-responsive breast cancer (22, 31), serum levels of PCBs and DDE were also compared based on the cases' ER status. It has been suggested that serum levels of PCBs and DDE may be artifactually increased in late-stage patients because of mobilization of energy from fat stores (32); therefore, we divided the cases into early (stages 0, I, and II) and later stages (stages III and IV), and each case category was compared with the control group. An earlier study suggested that chemotherapy might increase the serum level of PCBs, but not DDE (33). Therefore, we compared controls with breast cancer patients based on type of treatment and the elapsed time between start of treatment and the time that blood samples were drawn. For PCBs, we also examined the association based on PCB structural and biological-activity groups as proposed by Wolff *et al.* (34). Nine PCBs were grouped into three groups: (*a*) potentially estrogenic and weak phenobarbital inducer (congener 187); (*b*) potentially antiestrogenic and dioxin-like (congeners 74, 118, 138, 156, and 170); and (*c*) phenobarbital, *CYP1A*, and *CYP2B* inducers (congeners 153, 180, and 183).

A linear logistic regression model was used to estimate the exposure and disease association and to adjust for potential confounders. We divided the serum levels of total PCBs and DDE into tertiles or quartiles based on the frequency distribution of the controls. Variables included in the final model were age (<47, 47–52, 53–63, ≥64 years), BMI (<21, 21–24.9, ≥25 kg/m^2), age at menarche (<13, 13–14, ≥15 years), lifetime months of lactation (0, 1–10, ≥11), age at first full-term pregnancy (nulliparous, <20, 20–25, ≥26 years), number of live births (0, 1–3, ≥4), lifetime months of hormone replacement therapy (0, 1–71, ≥72), dietary fat intake in g/day (<46, 46–71, ≥72, unknown), family breast cancer history (including mother, sisters, and daughters), income 10 years before disease diagnosis or interview (<$20,000, $20,000–24,999, ≥$25,000, or unknown), and race (white, black, and other). The study site (Tolland County or New Haven County) was also adjusted when the risk was assessed for the entire study population. Maximum likelihood estimates of the parameters were obtained using SAS (35). Tests for trend were conducted using a likelihood ratio statistic in a logistic regression model. Breast cancer risk was also assessed based on menopausal, parity, and lactation status and by study site.

Results

As shown in Table 1, cases were slightly older than controls despite the attempt at matching; therefore, age was controlled for in all subsequent analyses. Women with a later age at first full-term pregnancy showed borderline significantly increased risk. Compared with those less than age 20 at first full pregnancy, the OR was 1.5 (95% CI, 0.9–2.3) for those having first full-term pregnancy at ages 20–25. The OR was 1.5 (95% CI, 0.9–2.5) for those having first full-term pregnancy at ages 26 and over. A history of lactation was associated with a nonsignificantly reduced risk compared with those who never lactated. A history of having a first-degree relative with breast cancer was associated with a nonsignificantly increased risk of breast cancer (OR = 1.3; 95% CI, 0.9–1.8). Dietary fat intake at the second tertile showed an increased risk (OR = 1.4; 95% CI, 1.0–1.9).

Table 1 Selected characteristics of breast cancer cases and controls in Connecticut

Characteristics	Cases (n = 475)	Controls (n = 502)	OR[a]	95% CI
Age (years)				
≤45	82	110	1.0	
>45	393	392	1.2	0.9–1.7
Age at menarche (years)				
≥15	57	54	1.0	
13–14	190	210	0.8	0.6–1.3
<13	227	237	0.9	0.6–1.3
Unknown	1	1		
Age at first full pregnancy (years)				
<20	42	55	1.0	
20–25	211	204	1.5	0.9–2.3
≥26	162	165	1.5	0.9–2.5
Nulliparous	60	78	1.2	0.7–2.1
Lifetime lactation (months)				
0	301	297	1.0	
1–10	94	111	0.8	0.5–1.1
≥11	80	94	0.8	0.6–1.2
Family breast cancer history				
No	391	429	1.0	
Yes	84	73	1.3	0.9–1.8
BMI (kg/m^2)				
<21.0	67	84	1.0	
21.0–24.9	183	205	1.1	0.8–1.6
≥25.0	225	213	1.2	0.8–1.8
Fat intake (g/day)				
<46	138	177	1.0	
46–71	175	166	1.4	1.0–1.9
≥72	150	152	1.3	0.9–1.7
Unknown	12	7	2.7	1.0–7.2
Annual income ($)				
<20,000	243	256	1.0	
20,000–24,999	40	46	0.9	0.5–1.4
≥25,000	73	101	0.8	0.5–1.1
Unknown	119	99	1.2	0.9–1.7
Race				
White	430	461	1.0	
Black	31	28	1.2	0.7–2.1
Other	14	13	1.1	0.5–2.4

[a] ORs for each selected characteristic were adjusted for all other selected characteristics listed in Table 1.

We found little difference with regard to these characteristics between the two control groups. For example, the mean age at menarche was 12.6 years for controls recruited from YNHH and 12.5 years for controls recruited from Tolland County. The mean age at first live birth was 23.5 years for YNHH and 24.0 years for Tolland County control subjects. The mean number of pregnancies was 3.3 for YNHH and 3.4 for Tolland County controls. The mean lifetime months of lactation among those who lactated was 13.5 months for YNHH and 12.9 months for Tolland County controls. The mean BMI was 25.9 kg/m^2 for YNHH and 25.6 kg/m^2 for Tolland County controls. The only baseline characteristic showing a slight difference between the control groups was family breast cancer history. For subjects recruited from New Haven County, the prevalence

Table 2 Age- and lipid-adjusted geometric means for PCBs and DDE among breast cancer cases and controls by study site

Variable	*n*	DDE (ppb)			PCBs (ppb)		
		Mean	95% CI	P^a	Mean	95% CI	P^a
All subjects							
Cases	475	460.1	423.1–500.5	0.89	733.1	706.3–761.0	0.46
Controls	502	456.2	420.5–494.9		747.6	721.0–775.1	
Tolland County							
Cases	149	489.6	424.9–564.0	0.17	843.8	776.0–917.5	0.73
Controls	155	425.9	370.8–489.1		861.3	793.5–934.8	
New Haven County							
Cases	326	451.4	407.0–500.7	0.44	687.4	662.6–713.1	0.42
Controls	347	477.8	432.2–528.2		702.1	677.6–727.5	

[a] *P* for geometric mean difference between cases and controls.

Table 3 Age- and lipid-adjusted geometric means for DDE and PCBs for controls and for cases according to stage at diagnosis and type of treatment

Variable	*n*	DDE (ppb)			PCBs (ppb)		
		Mean	95% CI	P^a	Mean	95% CI	P^a
Stage at diagnosis							
Controls	502	456.2	420.5–495.5		747.6	721.2–775.1	
Cases							
0–II	389	455.9	415.5–500.2	0.99	719.8	690.9–749.9	0.17
III–IV	20	402.1	267.1–605.2	0.57	638.9	533.5–765.1	0.57
Unknown	66	506.2	404.2–633.8	0.37	851.4	771.0–940.1	0.37
Type of treatment							
Controls	347	497.2	449.3–550.1		699.9	675.2–725.5	
Cases							
Chemotherapy	41	632.4	471.7–848.0	0.13	676.2	609.3–750.4	0.51
Surgery	154	442.1	380.5–513.6	0.21	699.2	662.9–737.4	0.90
Combination	86	483.6	395.3–591.5	0.79	678.0	631.2–728.3	0.52
Other	45	434.0	329.3–573.7	0.37	664.3	601.9–733.1	0.34

[a] *P* for geometric mean difference between cases and controls.

of family breast cancer history was 18.1% for cases and 15.3% for controls. For subjects recruited from Tolland County, the prevalence was 16.8% for cases and 12.9% for controls.

The age- and lipid-adjusted mean serum DDE level (Table 2) among the cases (460.1 ppb) was comparable to the level among the controls (456.2 ppb). Further stratification by study site (Tolland County or New Haven County) also showed no significant difference in serum DDE levels between the cases and controls. The age- and lipid-adjusted mean serum total PCBs levels were also quite comparable between all cases and all controls and among both study sites, whereas the serum levels of PCBs for cases (843.8 ppb) and controls (861.3 ppb) from Tolland County were higher than those for cases (687.4 ppb) and controls (702.1 ppb) from New Haven County (Table 2).

As shown in Table 3, the age- and lipid-adjusted geometric mean serum levels of DDE (402.1 ppb) and PCBs (638.9 ppb) for 20 patients with later stage disease (stages III and IV) were insignificantly lower than those (455.9 ppb for DDE and 719.8 ppb for PCBs) for 389 patients with early stage disease (*in situ*, stages I and II). Comparison of the means for the controls with each of the three categories of patients (early stage, later stage, and stage unknown), using the general linear model, showed no significant differences for either serum levels of PCBs or DDE (Table 3).

We further examined the data by type of treatment for 673 subjects recruited from YNHH, where detailed treatment information was available (Table 3). We found no significant differences between controls and the various treatment groups for mean serum levels of DDE or PCBs. Although the serum DDE level for the 41 patients who received chemotherapy was insignificantly higher than that of the controls, it did not suggest a trend with time elapsed since chemotherapy. The mean serum DDE levels for patients with chemotherapy were 1118.1, 873.5, 403.9, 504.6, 909.3, 327.0, and 952.2 ppb, respectively, for those with blood drawn 1, 2, 3, 4, 5, 6, or >6 months after treatment. The mean serum PCB levels for patients with chemotherapy, which was suggested as a factor that might increase serum PCBs, also did not show a trend with time elapsed since chemotherapy.

We also found no significant differences in mean serum levels of DDE or PCBs between controls and cases based on ER status for the 673 subjects recruited from YNHH (Table 4). The serum levels of PCBs and DDE were quite similar between the patients with ER-positive or -negative diseases. We also found little difference in serum levels of PCBs and DDE based on type of benign breast disease (nonproliferative, proliferative benign breast diseases, and normal controls) and different histological types of breast cancer (ductal and lobular carcinoma; data not shown).

The age- and covariate-adjusted OR was 0.96 (95% CI, 0.67–1.36) for DDE when the third tertile was compared with the lowest tertile for 475 breast cancer cases compared with the

Table 4 Age- and lipid-adjusted geometric means for DDE and PCBs among controls and breast cancer cases by ER status

Variable	*n*	DDE (ppb)			PCBs (ppb)		
		Mean	95% CI	*P*[a]	Mean	95% CI	*P*[a]
Controls	347	477.9	432.3–528.3		702.1	677.6–727.6	
Cases							
ER+	163	435.2	375.6–504.2	0.28	680.2	645.5–716.6	0.33
ER−	140	453.9	387.7–531.4	0.60	700.2	662.1–740.5	0.94
Unknown	23	563.5	381.9–831.5	0.42	661.1	575.9–759.0	0.43

[a] *P* for geometric mean difference between cases and controls.

Table 5 Risk of breast cancer associated with lipid-adjusted serum levels of DDE

Serum level (ppb)	Cases ($n = 475$)[a]	Controls ($n = 502$)[a]	OR[b] (95% CI)	OR[c] (95% CI)
All subjects				
<295.0	139	166	1.00	1.00
295.0–660.0	157	166	1.09 (0.79–1.50)	1.05 (0.76–1.47)
>660.0	179	170	1.11 (0.80–1.54)	0.96 (0.67–1.36)
P for trend			0.42	0.58
Parous women who ever breast fed				
<295.0	70	87	1.00	1.00
295.0–660.0	48	65	0.88 (0.54–1.45)	0.87 (0.51–1.49)
>660.0	56	53	1.14 (0.67–1.93)	1.07 (0.60–1.93)
P for trend			0.45	0.85
Parous women who never breast fed[d]				
<295.0	53	58	1.00	1.00
295.0–660.0	83	69	1.28 (0.78–2.10)	1.19 (0.71–2.01)
>660.0	103	91	1.11 (0.68–1.80)	0.96 (0.57–1.62)
P for trend			0.85	0.59
Nulliparous women[e]				
<295.0	16	21	1.00	1.00
295.0–660.0	24	31	1.05 (0.45–2.46)	0.45 (0.15–1.28)
>660.0	20	26	1.20 (0.46–3.12)	0.59 (0.16–2.16)
P for trend			0.30	0.74

[a] Total number of cases or controls.
[b] Adjusted only for age (<47, 47–52, 53–63, ≥64 years).
[c] Additional adjustments include BMI (<21, 21–24.9, ≥25 kg/m^2), age at menarche (<13, 13–14, ≥15 years), lifetime months of lactation (0, 1–10, ≥11), age at first full-term pregnancy (nulliparous, <20, 20–25, ≥26 years), number of live births (0, 1–3, ≥4), lifetime months of hormone replacement therapy (0, 1–71, ≥72), dietary fat intake in g/day (<46, 46–71, ≥72, unknown), family breast cancer history, income (<$20,000, $20,000–24,999, ≥$25,000, or unknown), race (white, black, and other), and study site.
[d] Not adjusted for lactation.
[e] Not adjusted for lactation, age at first full-term pregnancy, and number of live births.

502 controls (Table 5). The test for trend also was not significant ($P = 0.58$). Further stratification by parity and lactation status showed no significant association with serum DDE levels among nulliparous women or parous women, with or without lactation histories. There was also no association between breast cancer risk and serum PCB levels for all subjects by parity and lactation status (Table 6). Further stratification by study site and menopausal status reached the same conclusion as the overall analyses (data not shown).

The age- and lipid-adjusted geometric mean serum levels for each of the three structure-activity congener groups as proposed by Wolff *et al.* (34) were comparable between the cases and controls. For group 1, the geometric mean was 66.6 ppb for cases and 65.2 for controls ($P = 0.25$). For group 2, the geometric mean was 308.3 ppb for cases and 307.4 ppb for controls ($P = 0.87$). For group 3, the geometric mean was 274.9 ppb for cases and 272.7 ppb for controls ($P = 0.75$). The association between risk of breast cancer and congener groups also was not statistically significant, as shown in Table 7. Although a 35–45% nonsignificant higher risk was observed for the third quartile for both congener groups 1 and 2, there was no clear increasing trend with increasing serum levels for these congener groups. None of the linear trend tests were statistically significant (Table 7).

An assessment of the joint effects for DDE and PCBs did not show significant effect modification for the two factors on the risk of female breast cancer under the logistic model. A likelihood ratio statistic of 1.01 ($df = 1$; $P = 0.31$) was observed for the interaction between DDE and PCBs based on the actual measures, and similar conclusions were also apparent when categorical measures were used for the assessment. Similar results were also obtained when DDE and PCB were both included in a model together, indicating that collinearity did not account for the absence of an effect in the multivariate analyses.

Discussion

Our study did not find an overall association between total serum PCBs and DDE and risk of female breast cancer. We also did not find a significant difference in risk associated with exposure to PCBs or DDE by parity and lactation status, ER status, and menopausal status. Analysis by three structural-

Table 6 Risk of breast cancer associated with lipid-adjusted serum levels of PCBs

Serum level (ppb)	Cases (n = 475)[a]	Controls (n = 502)[a]	OR[b] (95% CI)	OR[c] (95% CI)
All subjects				
<604.0	155	164	1.00	1.00
604.0–800.0	160	167	1.02 (0.75–1.39)	1.04 (0.76–1.45)
>800.0	160	171	0.96 (0.70–1.31)	0.95 (0.68–1.32)
P for trend			0.44	0.41
Parous women who ever breast fed				
<604.0	61	66	1.00	1.00
604.0–800.0	61	75	0.89 (0.55–1.45)	0.94 (0.56–1.58)
>800.0	52	64	0.86 (0.52–1.43)	0.97 (0.56–1.68)
P for trend			0.40	0.89
Parous women who never breast fed[d]				
<604.0	76	71	1.00	1.00
604.0–800.0	76	67	1.06 (0.66–1.70)	1.01 (0.62–1.66)
>800.0	87	80	1.00 (0.63–1.57)	0.94 (0.58–1.55)
P for trend			0.68	0.65
Nulliparous women[e]				
<604.0	17	26	1.00	1.00
604.0–800.0	23	25	1.31 (0.57–3.00)	1.52 (0.56–4.16)
>800.0	20	27	1.06 (0.45–2.49)	0.77 (0.27–2.21)
P for trend			0.66	0.80

[a] Total number of cases or controls.
[b] Adjusted only for age (<47, 47–52, 53–63, ≥64 years).
[c] Additional adjustments include BMI (<21, 21–24.9, ≥25 kg/m^2), age at menarche (<13, 13–14, ≥15 years), lifetime months of lactation (0, 1–10, ≥11), age at first full-term pregnancy (nulliparous, <20, 20–25, ≥26 years), number of live births (0, 1–3, ≥4), lifetime months of hormone replacement therapy (0, 1–71, ≥72), dietary fat intake in g/day (<46, 46–71, ≥72, unknown), family breast cancer history, income (<$20,000, $20,000–24,999, ≥$25,000, or unknown), race (white, black, and other), and study site.
[d] Not adjusted for lactation.
[e] Not adjusted for lactation, age at first full-term pregnancy, and number of live births.

Table 7 ORs for breast cancer associated with serum levels of PCBs by congener group

Serum level (ppb)	Cases (n = 475)[a]	Controls (n = 502)[a]	OR[b] (95% CI)	OR[c] (95% CI)
Group 1: Potentially estrogenic				
<52.6	95	114	1.00	1.00
52.6–61.5	104	115	1.16 (0.79–1.70)	1.14 (0.77–1.70)
61.6–78.9	160	144	1.42 (0.99–2.02)	1.45 (0.99–2.11)
≥79.0	116	129	1.26 (0.85–1.85)	1.32 (0.87–2.01)
P for trend			0.66	0.71
Group 2: Potentially antiestrogenic				
<247.0	112	129	1.00	1.00
247.0–292.9	117	115	1.21 (0.84–1.73)	1.22 (0.84–1.77)
293.0–379.9	144	131	1.33 (0.94–1.89)	1.35 (0.94–1.93)
≥380.0	102	127	0.96 (0.67–1.39)	0.96 (0.65–1.40)
P for trend			0.95	0.95
Group 3: Phenobarbital, CYP1A, and CYP2B inducers				
<212.0	127	124	1.00	1.00
212.0–256.5	115	129	0.88 (0.62–1.26)	0.87 (0.60–1.25)
256.6–332.9	98	124	0.78 (0.54–1.12)	0.78 (0.54–1.15)
≥333.0	135	125	1.03 (0.72–1.46)	1.02 (0.71–1.47)
P for trend			0.89	0.85

[a] Total number of cases or controls.
[b] Adjusted only for age (<47, 47–52, 53–63, ≥64 years).
[c] Additional adjustments include BMI (<21, 21–24.9, ≥25 kg/m^2), age at menarche (<13, 13–14, ≥15 years), lifetime months of lactation (0, 1–10, ≥11), age at first full-term pregnancy (nulliparous, <20, 20–25, ≥26 years), number of live births (0, 1–3, ≥4), lifetime months of hormone replacement therapy (0, 1–71, ≥72), dietary fat intake in g/day (<46, 46–71, ≥72, unknown), family breast cancer history, income (<$20,000, $20,000–24,999, ≥$25,000, or unknown), race (white, black, and other), and study site.

activity congener groups also showed no significant difference in age- and lipid-adjusted geometric mean serum levels for each of the congener groups between the cases and controls. The risk of breast cancer was not significantly increased with any of the congener groups, although a 35–45% nonsignificant higher risk was observed for the third quartile for both congener groups 1 (which was assumed to have potential estrogenic activity) and 2 (which was assumed to have potential antiestrogenic activity). There is no clear increasing trend with increasing serum levels for these congener groups. There was no significant effect modification between DDE and PCBs on the risk of breast cancer in this study.

Many investigators have discussed the estrogenicity of various organochlorine compounds, including PCBs and DDT congeners (36–40). Some have suggested that PCBs and DDE, through their estrogenic actions, might increase breast cancer risk (12, 21, 22). Most of the estrogenic environmental compounds, however, are weak estrogens with a potency as low as 100,000-fold lower than that of natural estrogen, 17β-estradiol. *p,p′*-DDT and *p,p′*-DDE generally are considered nonestrogenic, whereas *o,p′*-DDT and *o,p′*-DDE are considered estrogenic, although their estrogenic activities are thousands of times lower than that of estradiol (36, 37). The hypothesis that relatively low exposure to weakly estrogenic organochlorine pesticides would significantly affect the risk of breast cancer is also difficult to reconcile with the inconsistent associations of more potent exogenous estrogens, such as postmenopausal hormone replacement therapy and oral contraceptive use (41, 42), with breast cancer.

In interpreting the results from the present study, however, several potential limitations need to be considered as discussed below. One of the potential limitations is the use of blood as the study medium. Organochlorine levels are generally lower in blood, which hampers evaluation of the association between breast cancer risk and specific PCB congeners. However, studies have suggested a good correlation between serum and adipose tissue levels of DDE and PCBs (43). The study by Moysich *et al.* (25) reported an OR of 3.6 (95% CI, 1.1–8.6) for moderately chlorinated PCBs when the third tertile was compared with the lowest among postmenopausal women who had never lactated, whereas no such increase in risk was seen for more highly chlorinated PCBs. Thus, the effect of individual or groups of PCBs as risk factors for breast cancer merits further investigation.

Another potential limitation concerns the use of patients with benign breast disease as controls in New Haven County and population-based controls in Tolland County. However, it does not seem likely that the lack of association between PCBs and DDE and breast cancer risk in our study can be attributed entirely to the inclusion of benign breast disease patients as part of the control group. As described earlier, we found little difference between the two control groups regarding baseline characteristics that might affect both body burden of organochlorine compounds and breast cancer risk, such as lifetime months of lactation, age at first live birth, number of pregnancies, and BMI. Univariate and multivariate analyses by study site also reached the same conclusions as the combined analyses. A positive association between DDE and PCBs and female breast cancer risk came from studies using patients with benign breast diseases as controls (21, 22).

Another concern is the potential for selection bias because of different participation rates for the cases and controls, ranging from 61% for controls in Tolland County to 77% for cases recruited from YNHH. If refusal, through potentially complex and poorly understood mechanisms underlying human behavior, is associated with both exposure and the disease of interest, a potential bias could be introduced. For example, if breast cancer cases with heavy exposure to PCBs or DDE were indeed more likely to refuse to participate in the study, refusal to participate in this study then may be partially responsible for the lack of association observed in this study.

It is also a concern that serum levels of PCBs and DDE in the case group may be affected by the disease process. Particularly, the serum levels of these compounds for late-stage patients may be artifactually increased because of mobilization of energy from fat stores (32). In our study, only 20 breast cancer patients were diagnosed with stage III/IV disease, and exclusion of these patients from the study did not result in any material change to the conclusions. A recent follow-up study (14) also does not support the hypothesis that disease stage at diagnosis significantly impacts serum levels of DDE and PCBs.

Gammon *et al.* (33) recently reported that treatment has no major impact on serum levels of DDE or PCBs after adjustment for lipid levels. In that study, the correlation coefficients for lipid-adjusted pretreatment and posttreatment levels of DDE and PCBs varied little across treatment groups, with the exception of chemotherapy, which showed some increase in blood levels for PCBs but not DDE. In our study, the age- and lipid-adjusted mean serum level of PCBs for patients who had received chemotherapy was slightly lower than the level for patients receiving surgery alone. This was also quite comparable to the mean for those receiving other treatments and the mean for the controls. The time between treatment and blood drawing had no consistent effect on serum levels of either PCBs or DDE. It should be pointed out that these comparisons do not rule out the possibility that treatment has not affected serum levels, because no data on pretreatment levels were available.

In conclusion, the risk of breast cancer associated with PCBs and DDE was systematically examined by parity and lactation status, ER status, and menopausal status in this study. The results do not support the hypothesis that DDE and PCBs increase the risk of female breast cancer as encountered through environmental exposure. However, the effect of individual or groups of PCBs as risk factors for breast cancer merits further investigation.

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